Molecular targeted therapy of glioblastoma

Le Rhun, Emilie ; Preusser, Matthias ; Roth, Patrick ; Reardon, David A ; van den Bent, Martin ; Wen, Patrick ; Reifenberger, Guido ; Weller, Michael

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Cover Letter

Cancer Treatment Reviews Zurich, August 24, 2019

Dear Editors

Please consider for publication as an Invited Review in *Cancer Treatment Reviews* the enclosed manuscript entitled " **Molecular Targeted Therapy of Glioblastoma** ".

Thank you very much for your consideration

Sincerely

Michael Weller, MD

Highlights

- Most glioblastomas are not “single pathway” diseases easily amenable to targeted therapy.
- The core pathways altered in glioblastoma are challenging for targeted drug design.
- Molecular genetic profiling, e.g. by large-scale DNA and RNA sequencing, may identify druggable molecular alterations in subsets of glioblastomas.

Conflicts of interest

ELR has received research grants from Mundipharma and Amgen and honoraria for lectures or advisory board participation from Abbvie, Daiichi Sankyo, Mundipharma and Novartis.

MP has received honoraria for lectures, consultation or advisory board participation from the following for-profit companies: Bayer, Bristol-Myers Squibb, Novartis, Gerson Lehrman Group (GLG), CMC Contrast, GlaxoSmithKline, Mundipharma, Roche, Astra Zeneca, AbbVie, Lilly, Medahead, Daiichi Sankyo, Merck Sharp & Dome. The following for-profit companies have supported clinical trials and contracted research conducted by MP with payments made to his institution: Böhringer-Ingelheim, Bristol-Myers Squibb, Roche, Daiichi Sankyo, Merck Sharp & Dome, Novocure, GlaxoSmithKline, AbbVie.

PR has received honoraria for advisory boards and lectures from BMS, Roche, MSD, Novartis, Novocure, Covagen, Virometix and Molecular Partners.

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Molecular targeted therapy of glioblastoma

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Abstract

Glioblastomas are intrinsic brain tumors thought to originate from neuroglial stem or progenitor cells. More than 90% of glioblastomas are isocitrate dehydrogenase (IDH)-wildtype tumors. Incidence increases with age, males are more often affected. Beyond rare instances of genetic predisposition and irradiation exposure, there are no known glioblastoma risk factors. Surgery as safely feasible followed by involved-field radiotherapy plus concomitant and maintenance temozolomide chemotherapy define the standard of care since 2005. Except for prolonged progression-free, but not overall survival afforded by the vascular endothelial growth factor antibody, bevacizumab, no pharmacological intervention has been demonstrated to alter the course of disease. Specifically, targeting cellular pathways frequently altered in glioblastoma, such as the phosphoinositide 3-kinase (PI3K)/protein kinase B (AKT)/mammalian target of rapamycin (mTOR), the p53 and the retinoblastoma (RB) pathways, or epidermal growth factor receptor (*EGFR*) gene amplification or mutation, have failed to improve outcome, likely because of redundant compensatory mechanisms, insufficient target coverage related in part to the blood brain barrier, or poor tolerability and safety. Yet, uncommon glioblastoma subsets may exhibit specific vulnerabilities amenable to targeted interventions, including, but not limited to: high tumor mutational burden, *BRAF* mutation, neurotrophic tryrosine receptor kinase (*NTRK*) or fibroblast growth factor receptor (*FGFR*) gene fusions, and *MET* gene amplification or fusions. There is increasing interest in targeting not only the tumor cells, but also the microenvironment, including blood vessels, the monocyte/macrophage/microglia compartment, or T cells. Improved clinical trial designs using pharmacodynamic endpoints in enriched patient populations will be required to develop better treatments for glioblastoma.

Introduction

Glioblastoma is an intrinsic brain tumor that may occur at any age and is thought to originate by genetic alterations affecting neuroglial stem or progenitor cells [1]. Incidence increases steadily with age. Median age at diagnosis is in the range of 65 years, and males are approximately 1.7-fold more often affected than females. Young age and good performance status are therapy-independent positive prognostic factors.

The updated WHO classification from 2016 [2] separates two major types of glioblastoma based on mutations in the isocitrate dehydrogenase (IDH) 1 or 2 genes, with IDH-wildype glioblastomas accounting for more than 90% of the cases. Histologically, glioblastomas are mitotically active tumors characterized by microvascular proliferation or necrosis or both as distinctive morphologic features of WHO grade IV. According to recent specifications of the consortium to inform molecular and practical approaches to CNS tumor taxonomy (cIMPACT) [3], molecular diagnostic detection of glioblastoma-associated genetic alterations, specifically epidermal growth factor receptor (*EGFR*) gene amplification, combined whole chromosome 7 gain and whole chromosome 10 loss (+7/−10), or telomerase reverse transcriptase (*TERT)* promoter mutation also allow for the diagnosis of IDH-wildtype glioblastoma despite the absence of WHO grade IV histological features. In contrast, astrocytic gliomas carrying an IDH mutation also may exhibit typical histological features of glioblastoma, but typically are associated with a more favorable clinical course and hence may no longer be classified as glioblastoma in up-coming revisions of the WHO classification. This review thus is focussed on the common IDH-wildtype tumors. The 2016 WHO classification [2] has maintained giant

cell glioblastoma and gliosarcoma as histological variants of IDH-wildtype glioblastoma, while, epithelioid glioblastoma was newly introduced as a provisional variant characterized by $BRAF^{V600E}$ mutation in up to 50% of cases [4], although more recent data did not identify distinct molecular profiles in these tumors [5]. While assigning a tumor to any of these variants has no clinical relevance in terms of tailoring standard treatment today [6], detection of $BRAF^{V600E}$ mutation in malignant gliomas including epithelioid glioblastomas may assume clinical relevance in terms of targeted therapy with mutant BRAF inhibitors (see below).

Besides histological variants, large-scale genetic and epigenetic profiling studies allowed for the distinction of several molecular subgroups of IDH-wildtype glioblastomas that are characterized by distinct DNA methylation patterns associated with characteristic mutational and expression profiles [7] [8] [9]. Up to seven molecular subgroups of IDH-wildtype glioblastoma have been identified by DNA methylation profiling, with the receptor tyrosine kinase (RTK) 1 and 2 subgroups as well as the mesenchymal subgroup being most common [9]. To date, distinction of these molecular subgroups is of limited relevance in terms of treatment, but this may change when future studies reveal subgroup-specific therapeutic vulnerabilities.

Current standard of care

Surgery as safely feasible followed by involved field radiotherapy in combination with concomitant and up to six maintenance cycles of temozolomide chemotherapy constitute the standard of care for the majority of patients with newly diagnosed glioblastoma [6] [10]. Except for tumor-treating fields [11], no other therapeutic intervention has been shown to prolong overall survival in the newly diagnosed setting.

All glioblastomas eventually progress, and standards of care at recurrence are less well defined. A small proportion of patients with localized recurrence are offered second surgery or re-irradiation, but neither of these interventions has been shown to prolong survival. Another course of alkylating chemotherapy, mostly the nitrosourea compound, lomustine, is the most broadly used intervention at recurrence and also constitutes the control arm in recent randomized trials in recurrent glioblastoma [12]. Yet, neither lomustine nor any other systemic treatment nor tumor-treating fields [13] have been shown to be superior to placebo or best supportive care in a randomized trial.

Targeted therapy for glioblastoma: focus on *MGMT* promoter methylation

Benefit from alkylating agent chemotherapy in glioblastoma is largely limited to patients whose tumors show aberrant CpG methylation of the promoter region of the O^6-methylguanine DNA methyltransferase (*MGMT*) gene. *MGMT* promoter methylation has been established as a predictive biomarker for benefit from temozolomide, although firmly only in the newly diagnosed setting [14] [15]. The AVAREG trial assessing fotemustine and bevacizumab appeared to confirm this predictive value also for a nitrosourea compound in the recurrent setting [16]. However, the REGOMA trial (see below), which also provided data on, albeit locally determined, MGMT status did not confirm a predictive value of MGMT promoter methylation for benefit from lomustine compared to regorafenib [17]. Since several large controlled trials with central MGMT testing have confirmed a strong prognostic role of MGMT promoter methylation in recurrent glioblastoma patients treated with temozolomide [18] or lomustine [12] [19], the potential predictive role of the MGMT status at recurrence needs to be further studied.

Drug repurposing

An emerging area of interest in cancer therapy, notably glioblastoma, is the repurposing of drugs that are already approved for other indications, based on assumptions of biochemical or metabolic features that might confer glioma cell sensitivity to such drugs. Importantly, retrospective analyses of survival associations of all these drugs need to be interpreted with caution and should be considered hypothesis-generating only. This is because it is difficult to control for comorbidities that led to the administration of these drugs, dosing was never standardized and not optimized for demonstrating anti-tumor activity, and data collection was typically limited to landmark analyses, but not cumulative dosing.

Anti-epileptic drugs

Despite decades of research, no conclusive evidence to support a survival-promoting activity of anti-epileptic drugs in brain tumor patients has been obtained. Mainly based on histone deacetylase inhibitory activity seen at high concentrations *in vitro* and putative differentiation-inducing activity, valproic acid has attracted particular interest. The observation of longer survival of patients treated with valproic acid in the EORTC 26981 trial [20] was not confirmed in an analysis of subsequent larger clinical trial populations [21], but the combination of valproic acid with temozolomide chemoradiotherapy continues to be explored [22]. Levetiracetam has been proposed to decrease the level of MGMT in glioblastoma [23], but no association with favorable outcome was seen in a large secondary analysis of clinical trial data [21]. Finally, after significant interest in the role of glutamatergic signaling affecting neurotransmission in the biology of glioblastoma, the initial excitement on the AMPA receptor antagonist, talampanel, subsided when it became clear that single arm phase II studies often

generate signals interpreted as promising, but are not predictive of success in randomized phase III settings [24] (see below).

Metformin

This anti-diabetic drug has been advanced as an adjunct to glioblastoma treatment because of presumed modulatory effects on metabolism, notably lowering glucose availability, suppression of insulin-like growth-factor signaling, and specifically inhibition of AMP-activated protein kinase. Yet, current evidence from pooled retrospective analyses of clinical trial data does not support the view that metformin warrants further study in glioblastoma [25].

Glioblastoma-intrinsic targets

The majority of clinical trial approaches focusing on glioblastoma-intrinsic targets address oncogenic signaling via tyrosine receptor kinases, cell cycle control and susceptibility to apoptosis induction.

Tyrosine kinase receptor pathways

Epidermal growth factor receptor (EGFR)

EGFR is one of most prominent oncogenes in IDH-wildtype glioblastoma. It is overexpressed in approximately 60% of tumors, and more than 40% exhibit *EGFR* gene amplification. A particular deletion mutation referred to as EGFRvIII or delta-EGFR is found in 25% of tumors and is of particular interest since it is

constitutively active and a potential neoantigen (Table 1). Numerous studies have failed to demonstrate single agent activity of EGFR tyrosine kinase inhibitors in unselected patient populations and small biological endpoint studies have raised concerns that these drugs may not be able to suppress pathway activity even if they reach the tumor tissue [26] [27]. Other approaches have used EGFR or EGFRvIII expression rather than EGFR pathway activity as a target: these include the vaccine, rindopepimut, which produced a survival signal when combined with bevacizumab in EGFRvIII-positive recurrent glioblastoma (NCT01498328), but failed in phase III (ACT IV) in newly diagnosed disease [28]. Similarly, the antibody drug conjugate, depatuxizumab mafodotin, consisting of the EGFR antibody ABT-806 linked to monomethyl auristatin F, appeared to be active in combination with temozolomide in recurrent *EGFR*-amplified glioblastoma [29], but showed no activity in newly diagnosed glioblastoma when combined with standard temozolomide chemoradiotherapy (NCT02573324). The ACT IV trial results showed a striking loss of EGFRvIII expression at recurrence in both groups of the trial, suggesting that EGFRvIII expression is unstable. These observations of unstable target expression also cast doubt on the likelihood of success of chimeric antigen receptor (CAR) T cells or bispecific T-cell engaging antibodies targeting EGFRvIII [30] [31]. In contrast, EGFR amplification appears to be maintained throughout the course of disease [32] [33].

PI3K/AKT/mTOR pathway

The PI3K/mTOR pathway is one of the almost inevitably altered molecular pathways in IDH-wildtype glioblastoma, as a consequence of loss of tumor suppressor phosphatase and tensin homolog on chromosome ten (PTEN) function, activating mutations in phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha

(*PIK3CA)*, the gene encoding the catalytic subunit p110 alpha (p110α), and in phosphoinositide-3-kinase regulatory subunit 1 (*PIK3R1)*, encoding the p85α regulatory subunit. Yet, like in other types of cancer, it has turned out to be challenging to translate this extensive knowledge on pathway alteration into clinical benefit, although there is a long tradition of targeting the PI3K pathway in glioblastoma.

As early as 2005, the lack of activity of the mTOR inhibitor, temsirolimus (CCL-779), was demonstrated at least when used as a single agent in recurrent glioblastoma [34]. It was also observed at this time that the toxicity profile was unfavorable in this patient population. More recently, in a similar setting but enrolling patients predicted to be enriched for active PI3K signaling, the pan-PI3K inhibitor, buparlisib was found to be essentially inactive. This trial included a phase 0-like biological endpoint cohort. A subset of patients exhibited reduced levels of phosphorylated AKT^{S473} in the tumor tissue as a direct read-out of PI3K inhibition, but suppression of down-stream $pS6^{S235/236}$ was less pronounced, demonstrating insufficient overall pathway inhibition by tolerable doses of buparlisib [35].

PI3K/mTOR pathway inhibition has also been explored in the newly diagnosed setting, either in combination with radiotherapy and temozolomide [36] or instead of temozolomide in patients with MGMT promoter-unmethylated glioblastoma [37]. Both trials concluded absence of an efficacy signal and both confirmed the relatively poor tolerability of mTOR inhibitors in this setting, at least compared with temozolomide.

In summary, it seems that the PI3K/AKT/mTOR pathway is overall too promiscuous, resulting in non-favorable tolerability and safety profiles upon pharmacological inhibition. Moreover, well designed clinical trials have indicated that currently available agents may be insufficient to inhibit the target adequately at doses that are tolerated by patients. One may speculate that even those tumors that are in principle sensitive

to target inhibition will rapidly develop escape pathways, circumventing the block of the PI3K pathway. Future efforts will have to employ more potent agents or will have to rely on combinatorial strategies, the development of which, however, probably requires a deeper understanding of how this pathway interacts with other pathways that are likely to be altered in glioblastoma.

MET

The *MET* gene encodes the receptor for hepatocyte growth factor, also known as scatter factor, and has been attributed a major role in the migration and invasiveness of glioma cells, notably in response to irradiation, inhibition of angiogenesis, and hypoxia [38]. It is commonly expressed in glioblastoma, often at high levels, but not a major mutational target (Table 1). Proof of concept for a role of MET amplification was obtained in a single patient with recurrent glioblastoma treated with crizotinib [39], but not in a second published case [40]. However, neutralizing the ligand appeared to have no impact on disease progression [41]. Blocking the receptor using the single arm antibody, onartuzumab [42] or cabozantinib, a tyrosine kinase inhibitor of MET, VEGFR2 and AXL, in patients without or with pre-exposure to anti-angiogenic agents [43] [44] resulted in a modest efficacy signal in recurrent disease. MET fusion genes have been detected in a minor subset of pediatric glioblastoma and have been linked to response to crizotinib in one patient [45].

Fibroblast growth factor receptor (FGFR)

FGFR are commonly expressed in glioblastomas, but relevance as a potential target for intervention is probably limited to patients with rare tumors exhibiting FGFR-TACC fusions [46]. Despite several ongoing efforts in this area, there is little data available in the public domain. One stable disease and one minor response were reported in two

patients with FGFR3-TACC3-positive recurrent glioblastoma patients treated with the oral pan-FGFR kinase inhibitor, erdafitinib [47].

BRAF mutation

BRAF, a member of the Raf family of kinases, feeds into the MAP kinase/ERK signaling pathway that promotes proliferation. Activating BRAF mutations, in particular the $BRAF^{V600E}$ missense mutation, are found in several tumor types and have been confirmed to be druggable molecular lesions, notably in metastatic melanoma [48]. Early studies and individual case reports indicated that glial tumors exhibiting BRAF mutations may also respond to pharmacological BRAF inhibition, although their efficacy seems modest at best in BRAFV600E-mutant glioblastoma [49] [50] [51].

Neurotrophic tyrosine receptor kinases (NTRK)

NTRK are encoded by three different genes, *NTRK1*, *NTRK2* and *NTRK3*. Genomic rearrangements in *NTRK* genes resulting in gene fusions may trigger activation of oncogenic TRK signaling. The prevalence of *NTRK* gene fusions in glioblastoma appears to be low [52], and larger studies are required to determine whether agents like larotrectinib or entrectinib are also active in NTRK fusion-positive glioblastoma [53].

Cell cycle control and apoptosis regulating pathways

The retinoblastoma (pRB) pathway

The pRB cell cycle control pathway is altered in the majority of IDH-wildtype glioblastomas due to homozygous *CDKN2A/B* deletion, *CDK4* or *CDK6* amplification, or *RB1* gene alterations (Table 1). This pathway is also required for the growth of

normal cells which may largely explain why its therapeutic targeting has remained challenging, not only in glioblastoma. The only completed phase II trial targeting this pathway in glioblastoma using palbociclib was disappointing [54], but patients enrolled in this trial represented a negative selection, as indicated by the short overall survival (Table 2).

TG02 is a multi-CDK inhibitor mainly targeting CDK9, but not CDK4/6, and acts independently of the cell cycle control pathway involving CDK4/6 to suppress the transcription of multiple short-lived survival genes. TG02 is currently being explored in clinical trials in recurrent and in newly diagnosed glioblastoma (NCT02942264, NCT03224104).

The p53 pathway

The *TP53* tumor suppressor gene is among the best and longest studied genes in glioblastoma. Since the key function of its gene product, p53, is to arrest cells in G0/1 or to trigger apoptosis in response to genotoxic stress, restoring p53 function by various approaches has been extensively studied. Yet, drugs aimed at facilitating refolding of mutant proteins into wildtype conformation have not been successful, but efforts focusing on neutralizing MDM2 and MDM4 for patients with glioblastomas that are deficient in p53 function as a consequence of MDM2 or MDM4 gene amplification are in progress ([55] and NCT03107780).

TERT promoter mutation

TERT promoter mutations are the most common molecular alteration in IDH-wildtype glioblastoma [8] [56]. They affect two mutation hotspots that create new Ets transcripton factor-binding sites and increase TERT transcription and thereby TERT activity supporting immortalization of tumor cells [57]. Nevertheless, TERT promoter

mutation has not become a major pharmacological target for cancer therapy yet. Eribulin, known as an inhibitor of tubulin polymerization, has been proposed to exert TERT inhibitory activity in glioblastoma models, justifying its clinical exploration [58].

Proteasome

The proteasome is a complex cellular machinery with several enzymatic activities involved in the degradation and recycling of cellular protein. Altered proteasome activity has emerged as a potential vulnerability of cancer cells and resulted in the development of drugs like bortezomib that have also been tested in recurrent glioblastoma [59] [60]. The brain-penetrant pan-proteasome inhibitor, marizomib, is currently undergoing phase III evaluation in newly diagnosed glioblastoma (NCT03345095).

Microenvironmental targets - Angiogenesis

Vascular endothelial growth factor (VEGF)

The detection of VEGF as a major mediator of angiogenesis in glioblastoma, at a time when conventional cancer treatments like radiotherapy and chemotherapy appeared to have reached their limits, triggered substantial efforts to establish anti-angiogenesis as a treatment paradigm in glioblastoma (Table 2). Based on high radiological response rates and encouraging survival outcomes in uncontrolled trials [61][62], bevacizumab achieved approval for recurrent glioblastoma in many parts of the world, but its effects on tumor biology and growth dynamics beyond what can be detected by neuroimaging remained controversial. It has remained challenging to identify

subgroups of glioblastoma patients that experience prolonged survival when treated with bevacizumab [63] [64], although there are glioblastoma models where VEGF may even be a survival factor [65]. Phase III trials in newly diagnosed and recurrent disease demonstrated prolonged PFS but consistently no OS benefit, although approximately 30% cross-over to bevacizumab was observed in these trials [12] [66] [67]. Other VEGF inhibitors such as cediranib also failed in randomized clinical trials [68].

Integrins

Integrins are cell surface molecules that integrate signals from cell to cell and extracellular matrix to cell and are involved in essential cellular processes such as adhesion, migration, invasion and angiogenesis. Specific subtypes of integrins are present in glioblastomas and their vasculature [69]. The results of a series of largely uncontrolled clinical trials were interpreted to justify a phase III trial on cilengitide in patients enriched for *MGMT* promoter methylated glioblastomas [70] [71]. Altogether, these trials did not provide any robust safety or efficacy signal using cilengitide as a lead compound for integrin inhibition, but efforts to employ tumor specific integrin expression as a target continue.

Transforming growth factor (TGF)-β

TGF-$\beta_{1/2}$ as the major representatives of a family of related proteins have been considered for decades as key molecules responsible for glioblastoma-mediated immunosuppression. However, this protein family is promiscuous in that it is involved in almost all physiological and pathological processes. Despite promising data

obtained with various modes of TGF-β inhibition in different animal models, clinical translation of TGF-β targeting using TGF-β2-specific antisense oligonucleotides [72] or tyrosine kinase inhibitors targeting TGF-β receptor II (ALK5) such as galunisertib [73] has remained unsuccessful. The introduction of TGF-β receptor inhibitors into the clinic has also remained challenging because of dose-limiting toxicity, e.g, to the cardiac and intestinal system, potentially preventing adequate target coverage in the tumor.

Programmed cell death protein (PD)-1

The development of neutralizing antibodies to immune checkpoint molecules has dominated the field of cancer immunotherapy. Notably antibodies preventing the engagement of PD-1 on T cells by is major ligand, PD-L1, expressed on tumor cells, or on host cells have attracted marked interest. Yet, despite major advances in the treatment of various solid tumors with immune checkpoint inhibition, e.g., melanoma or non-small cell lung cancer, immune checkpoint inhibition has not been successful in glioblastoma, with the exception of single cases of encouraging responses in patients with tumors with high mutational burden due to germ line mutations affecting DNA repair [74]. In contrast, large randomized clinical trials of nivolumab versus bevacizumab in recurrent glioblastoma (CheckMate 143) [75] or of nivolumab versus temozolomide, both in combination with radiotherapy, in MGMT promoter unmethylated newly diagnosed glioblastoma (CheckMate 498) have failed to improve survival.

Outlook

The failure of several targeted agents for glioblastoma in late clinical development illustrates that most glioblastomas are not even close to being a single pathway-driven disease that would be amenable to targeted therapy. There is a need for improved clinical trial design and an early inclusion of control arms in phase II settings that allow to arrive at meaningful go/no-go decisions for further clinical development. Furthermore, platform trials exploring multiple compounds in parallel would in theory accelerate drug development. EORTC has developed the SPECTA (Screening Cancer Patients for Efficient Clinical Trial Access) platform [76], but this approach has not yet been applied to glioblastoma. Innovative clinical trial concepts such as AGILE (Adaptive Global Innovative Learning Environment for Glioblastoma) and INSIGhT (Individualized Screening Trial of Innovative Glioblastoma Therapy) propose multiple arms that can be enlarged or terminated early in a dynamic manner according to their probability of success which requires continuous monitoring [77]. Such trials may speed drug development significantly, but their success will ultimately depend on whether active compounds are tested in well selected patients. Another, not mutually exclusive approach would assign patients to clinical trial arms based upon molecular profiling in real time. While such clinical trial concepts are overall innovative and promising, there are certain caveats [55]. Molecular testing needs to be done quickly enough to allow patient treatment in adequate time, algorithms to rank the molecular lesions found need to be in place, and pharmaceutical companies need to be motivated to collaborate in early phases of drug development. The only randomized effort to demonstrate a superiority of treatment allocation based on molecular testing over standard of care, the French SHIVA trial, comes from the non-neuro-oncology area and failed to demonstrate overall benefit [78], (but profiling techniques have made great progress and novel targets continue to be identified.

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Figure Legends

Figure 1. Candidate molecular pathways amenable to targeted interventions in glioblastoma.

Figure 2. Selected current strategies of targeted therapy for glioblastoma. A, EGFR; B, PI3K/AKT/mTOR; C, MET, D, FGFR; E, BRAF; F, NTRK; G, VEGF; H, Integrins; I, TGF-β.

Tables

Table 1. Candidate target molecules for molecular therapy of IDH wildtype glioblastoma

Target	Biological function	Significance in glioblastoma	Selected references
Glioblastoma-intrinsic targets			
Epidermal growth factor receptor (EGFR)	Proliferation, invasion, resistance to apoptosis induction	Amplification in 40-50%, including EGFRvIII mutation in half of these, 10-20% overexpression without amplification	Brennan et al. 2013 [8], Felsberg et al. 2017 [33]
PI3K/AKT/mTOR	Metabolism, proliferation, migration	Almost uniformly activated pathway due to loss of *PTEN* function or activating mutations in *PIK3CA* or *PIK3R1*	Brennan et al. 2013 [8]
MET	Migration, invasion, wound healing	Overexpression, rarely amplification (5%)	Brennan et al. 2013 [8], Kwak et al. 2015 [79]
FGFR	Proliferation	FGFR transforming acidic coiled-coil (TACC) gene fusions (3%) and very rarely activating mutations	Singh et al., 2012 [46], Di Stefano et al. 2015 [47]
BRAF	Proliferation	BRAFV600E mutations in up to 50% of epithelioid glioblastomas, otherwise rare	Korshunov et al. 2018 [5]
NTRK	Proliferation	NTRK gene fusions detected in 1-2%	Ferguson et al. 2018 [52]
CDK4/6 or CDKN2A/B or RB	Cell cycle progression	Pathway often disrupted, because of CDK4/6 amplification (15%), CDKN2A/B loss (50%) or RB mutation (10%)	Brennan et al. 2013 [8]
P53	Cell cycle progression and apoptosis induction	Mutation or deletion (35%) or neutralization by MDM2/4 amplification (20%)	Brennan et al. 2013 [8]
TERT	Proliferation, cellular longevity	Enhanced expression resulting from promoter	Brennan et al. 2013

		mutation (90%)	[8]
Microenvironmental targets			
Vascular endothelial growth factor (VEGF)	Blood vessel formation	Angiogenesis triggered by hypoxia, potentially survival factor for subsets of glioblastomas	Plate et al. 1994 [80], Szabo et al. 2016 [65]
Integrins	Adhesion, migration, adhesion, activation of TGF-β pathway	αv integrins are expressed in blood vessels and tumor cells	Roth et al. 2013 [69]
TGF-β	Pleiotropic cytokine involved in most biological processes	Immunosuppression, migration, invasion, angiogenesis	Frei et al. 2015 [81]
PD-L1	Immune checkpoint, limiting T cell activation	Expression levels in glioblastoma including tumor microenvironment remain controversial	Berghoff et al. 2015 [82], Nduom et al. 2016 [83]

Table 2. Selected phase II and III trials of targeted therapy in glioblastoma

Reference & & selected trials	**Intervention**	**Patient population (n)**	**Enrichment**	**Design**	**Primary endpoint**	**Response**	**PFS**	**OS**	**Conclusions**
Glioblastoma-intrinsic targets									
EGFR									
Van den Bent et al. 2009 [84]	Erlotinib	Recurrent, erlotinib (54) versus BCNU or TMZ (56)	None	Randomized phase II. open label	PFS-6	Erlotinib PR: 3.7% BCNU/TMZ PR: 9.6%	PFS-6 (%) Erlotinib 11.4 BCNU/TMZ 24 median PFS (months) erlotinib 1.8 BCNU/TMZ 2.4	Median OS (months) erlotinib 7.7 BCNU/TMZ 7.3	EGFR TKI do not provide benefit in unselected patients
Weller et al. 2017 [28]	Rindopepimut	Newly diagnosed, rindopepimut (371) versus control (374), plus TMZ/RT→TMZ	EGFRvIII expression	Randomized phase III, placebo-controlled	OS		Median PFS (months) rindopepimut 7.1 placebo 5.6	Median OS (months) rindopepimut 20.1 placebo 20.0	Rindopepimut is inactive in newly diagnosed disease
van den Bent et al., 2018 [29]	Depatuxizumab / ABT-414	Recurrent, ABT-414 plus TMZ (88) versus ABT-414 (86) versus TMZ or CCNU (86)	EGFR amplification	Randomized phase II, open label	OS	ABT-414 plus TMZ 5 PR ABT-414 2 PR TMZ/CCNU 1 PR	Median PFS (months) ABT-414 plus TMZ 3 ABT-414 1.9 TMZ/CCNU 2.0	Median OS (months) ABT-414 plus TMZ 9.6 ABT-414 7.9 TMZ/CCNU 8.2	ABT-414 may be active in combination with TMZ
PI3K/AKT/mTOR									
Chang et al. 2005 [34]	Temsirolimus	Recurrent (43)	None	Single arm phase II	PFS-6	2 PR	PFS-6 2%	ND	Temsirolimus is inactive as single agent
Ma et al. 2015 [36]	Everolimus	Newly diagnosed (104), plus TMZ/RT→TMZ	None	Single arm phase II	OS-12		median PFS 6.4 months	OS-12: 64% median OS: 15.8	Everolimus is not active in combination with

								months	TMZ/RT→TMZ
Wick et al. 2016 [37]	Temsirolimus	Newly diagnosed, temsirolimus plus RT (56) or TMZ/RT→TMZ (55)	None, MGMT promoter unmethylated	Randomized phase II, open label	OS-12		Median PFS (months) temsirolimus 5.4 TMZ 6.0	OS-12 Temsirolimus 70% TMZ 72% Median OS (months) Temsirolimus 14.8 TMZ 16.0	mTOR^{Ser2448} phosphorylation may be used for enrichment in further studies of mTOR inhibition
Wen et al. 2019 [35]	Buparlisib	Recurrent (50)	PI3K pathway activated	Single arm phase II	PFS-6	None	PFS-6 8%	9.8 months	Buparlisib is inactive as single agent
MET									
Wen et al. 2011 [41]	Rilotumumab	Recurrent (60), rilotumumab at 10 or 20 mg/kg	None	Single arm phase II	ORR	None	PFS (months) 10 mg/kg: 1.0 20 mg/kg: 1.0	OS (months) 10 mg/kg: 6.5 20 mg/kg: 5.4	Rilotumumab is inactive
Cloughesy et al. 2017 [42]	Onartuzumab	Recurrent, onartuzumab plus bevacizumab (64) or bevacizumab (65)	None	Randomized phase II, open label	PFS-6	onartuzumab plus bevacizumab 1 CR, 11 PR bevacizumab 3 CR, 11 PR	PFS-6 (months) onartuzumab plus bevacizumab 3.9 bevacizumab 2.9	OS (months) onartuzumab plus bevacizumab 8.8 bevacizumab 12.6	High tumor hepatocyte growth factor and lack of MGMT promoter methylation may predict benefit from MET inhibition
BRAF									
Kaley et al. 2018 [49]	Vemurafenib	BRAF-mutant glioma (24), including 6 glioblastomas	BRAF mutation	Single arm phase II	ORR	None, 3 SD in 6 patients			BRAF inhibition in gial brain tumors deserves further study
CDK4/6 or CDKN2A/B or RB									
Taylor et al. 2018	Palbociclib	Recurrent (22)	RB1-positive	Single arm	PFS-6		Median PFS 5	Median OS 15	Palbociclib is

[54]			by immune-histochemistry	phase II			weeks	weeks	inactive as single agent
Proteasome									
Friday et al. 2012 [60]	Bortezomib plus vorinostat	Recurrent (34)	None	Single arm phase II	PFS-6	1 PR	PFS-6: 0%	Median OS 3.2 months	No indication to further study this combination
Kong et al. 2018 [59]	Bortezomib	Recurrent (24)	None	Single arm phase II	OS		Median PFS 6.2 months	Median OS 19.1 months	Bortezomib warrants further study
Multi-kinase inhibition									
Lee et al. 2016 [85]	Vandetanib[1]	Newly diagnosed, TMZ/RT→TMZ (36) or TMZ/RT→TMZ plus vandetanib (70)	None	Randomized phase II, open label	OS		PFS (months) TMZ/RT→TMZ 6.2 TMZ/RT→TMZ plus vandetanib 7.7	OS (months) TMZ/RT→TMZ 15.9 TMZ/RT→TMZ plus vandetanib 16.6	Vandetanib is inactive
Wen et al. 2018 [44]	Cabozantinib[2]	Recurrent, without prior anti-angiogenic therapy (34 at 140 mg/d, 118 at 100 mg/d))	None	Single arm phase II	ORR	6 PR and 17 PR	PFS-6: 22.3% and 27.8%	Median OS 7.7 and 10.4 months	Cabozantinib is inactive as single agent
Cloughesy et al. 2018 [43]	Cabozantinib	Recurrent, with prior anti-angiogenic therapy (70)	None	Single arm phase II	ORR	3 PR	PFS-6: 8.5%	Median OS 4.6 months	Cabozantinib is inactive as single agent
Lombardi et al. 2018 [17]	Regorafenib[3]	Recurrent, regorafenib (59) versus lomustine (60)	None	Randomized phase II, open label	OS	Regorafenib 1 CR, 2 PR Lomustine 1 CR, 1 PR	PFS-6 Regorafenib 16.9% Lomustine 8.3%	OS (months) Regorafenib 7.4 Lomustine 5.6	Regorafenib warrants further study in glioblastoma
Microenvironmental targets									
VEGF									

Kreisl et al. 2009 [61]	Bevacizumab	Recurrent (48)	None	Single arm phase II	PFS-6	1 CR, 16 PR	PFS-6 29%	OS 7.2 months	Bevacizumab is active
Friedman et al. 2009 [62]	Bevacizumab	Recurrent, bevacizumab (85) or bevacizumab plus irinotecan (82)	None	Randomized phase II, open label	PFS-6 and ORR	Bevacizumab 1 CR, 23 PR bevacizumab plus irinotecan 2 CR, 29 PR	PFS-6 bevacizumab 43% bevacizumab plus irinotecan 50%	OS (months) bevacizumab 9.2 bevacizumab plus irinotecan 8.7	Bevacizumab is active
Chinot et al. 2014 [67]	Bevacizumab	Newly diagnosed, TMZ/RT→TMZ (463) or TMZ/RT→TMZ plus bevacizumab (458)	None	Randomized phase III, placebo-controlled	Investigator-assessed PFS and OS		PFS (months) control 6.2 bevacizumab 10.6	OS (months) control 16.7 bevacizumab 16.8	Bevacizumab does not prolong OS
Gilbert et al. 2014 [66]	Bevacizumab	Newly diagnosed, TMZ/RT→TMZ (317) or TMZ/RT→TMZ plus bevacizumab (320)	None	Randomized phase III, placebo-controlled	OS and PFS		PFS (months) control 7.3 bevacizumab. 10.7	OS control 16.1 bevacizumab 15.7 months	Bevacizumab does not prolong OS
Herrlinger et al. 2016 [86]	Bevacizumab	Newly diagnosed, TMZ/RT→TMZ (54) or RT plus bevacizumab plus irinotecan (116)	None, MGMT promoter unmethylated	Randomized phase II, open label	PFS		PFS-6: TMZ/RT→TMZ 43% RT plus bevacizumab plus irinotecan 79%	OS (months) TMZ/RT→TMZ 17.5 RT plus bevacizumab plus irinotecan 16.6	Trial results inconclusive
Taal et al. 2014 [19]	Bevacizumab	Recurrent, lomustine or bevacizumab or bevacizumab plus lomustine	None	Randomized phase II, open label	OS-9	ORR lomustine 5% bevacizumab 38% bevacizumab plus lomustine 34%	PFS-6 lomustine 13% bevacizumab 16% bevacizumab plus lomustine 41%	OS-9 lomustine 38% bevacizumab 43% bevacizumab plus lomustine 87%	Bevacizumab plus lomustine may be superior to monotherapy
Wick et al. 2017 [12]	Bevacizumab	Recurrent, bevacizumab plus lomustine (288) or	None	Randomized phase III, open-label	OS	Bevacizumab plus lomustine 5 CR, 103 PR	PFS (months) bevacizumab plus lomustine	OS (months) bevacizumab plus lomustine	Bevacizumab does not prolong OS

		lomustine (149)				lomustine 1 CR, 18 PR	4.2 lomustine 1.5	9.1 lomustine 8.6	
Wirsching et al. 2018 [64]	Bevacizumab	Newly diagnosed, hypofractionated RT plus bevacizumab (n=50) or RT alone (n=25)	Elderly patients (65 years or more), MGMT promoter unmethylated (after amendment)	Randomized phase II	OS	Bevacizumab plus RT 6 CR, 17 PR RT 4 CR, 3 PR	PFS-6 Bevacizumab plus RT 78% RT 28% PFS Bevacizumab plus RT 7.6 RT 4.8	OS (months) Bevacizumab plus RT 12.1 RT 12.2	Old age and absence of MGMT promoter methylation fail to predict improved OS with bevacizumab
De Groot et al. 2011 [87]	Aflibercept (VEGF trap)	Recurrent (42)	None	Single arm phase II	PFS-6	7 PR	PFS-6 8%	OS 9.1 months	Aflibercept is inactive
Batchelor et al. 2010 [88]	Cediranib	Recurrent (31)	None	Single arm phase II	PFS-6	8 PR	PFS 3.9 months	OS 7.6 months	Cediranib warrants further study
Batchelor et al. 2013 [68]	Cediranib	Recurrent, cediranib (131) or cediranib plus lomustine (129) or lomustine (65)	None	Randomized phase III, partially blinded	PFS	Cediranib 1 CR, 17 PR cediranib plus lomustine 2 CR, 19 PR lomustine 5 PR	PFS (months) cediranib 3.1 cediranib plus lomustine 4.2 lomustine 2.7	OS (months) cediranib 8.0 cediranib plus lomustine 9.4 lomustine 9.8	Cediranib is not superior to lomustine and does not prolong survival in combination
Integrins									
Reardon et al. 2008 [89]	Cilengitide	Recurrent, cilengitide high (40) or low (41) dose	None	Randomized phase II, open label	PFS-6	high 5 responses low 2 responses	PFS-6 high 15% low 10%	OS (months) high 9.9 low 6.5	Cilengitide is moderately active
Stupp et al. 2010 [70]	Cilengitide	Newly diagnosed, TMZ/RT→TMZ plus cilengitide (52)	None	Single arm phase II	PFS		PFS 8 months	OS 16.1 months	Cilengitide warrants further study
Nabors et al. 2012 [90]	Cilengitide	Newly diagnosed, TMZ/RT→TMZ plus cilengitide	None	Randomized phase II, open label	OS		PFS (months) high 9,3 low 9.5	OS (months) high 20.8 low 17.4	Cilengitide warrants further study

		high (48) or low (46) dose							
Stupp et al. 2014 [71]	Cilengitide	Newly diagnosed, TMZ/RT→TMZ without (273) or with cilengitide (272)	None, MGMT promoter methylated	Randomized phase III, open label	OS		PFS (months) control 7.9 cilengitide 10.6	OS 26.3 in both arms	Cilengitide is inactive
Nabors et al. 2015 [91]	Cilengitide	Newly diagnosed, TMZ/RT→TMZ without (89) or with cilengitide high (88) or low (88) dose	None, MGMT promoter unmethylated	Randomized phase II, open label	OS		PFS (months) Control 4.1 low 5.6 high 5.9	OS (months) control 13.4 low 16.3 high 14.5	Inconclusive study results
Angiopoietins									
Reardon et al. 2018 [92] 2018	Trebananib	Recurrent, trebananib (11) or trebananib plus bevacizumab (37)	None	Single arm phase II	PFS-6	Trebananib none trebananib plus bevacizumab 10 PR	PFS-6 Rebananib 0% trebananib plus bevacizumab 24%	OS (months) Trebananib 11.4 trebananib plus bevacizumab 9.5	Trebananib is inactive
PKC									
Wick et al. 2010 [93]	Enzastaurin	Recurrent, enzastaurin (266) or lomustine (92)	None	Randomized phase III, open label	PFS	enzastaurin 5 responses lomustine 4 responses	PFS (months) enzastaurin 1.5 lomustine 1.6	OS (months) enzastaurin 6.6 lomustine 7.1	Enzastaurin is inactive
Wick et al. 2013 [94]	Enzastaurin	Newly diagnosed (60)	None, MGMT promoter unmethylated	Single arm phase II	PFS-6		PFS-6 54%	OS 15 months	Enzastaurin is inactive
TGF-β									
Bogdahn et al. 2011 [72]	Trabedersen (TGF-β_2 antisense oligonucleotide)	Recurrent, Trabedersen high (34) or low (28) dose or TMZ/PCV (33)	None	Randomized phase II, open label	6 months tumor control rate	Trabedersen high 1 PR, low none, TMZ/PCV none	Tumor control rate Trabedersen high 12%, low 14%, TMZ/PCV 15%	OS (months) Trabedersen high 10.9, low 7.3, TMZ/PCV 10	Trabedersen should be further evaluated (at the time)
Brandes et al. 2016	Galunisertib	Recurrent,	None	Randomized	OS	galunisertib	PFS (months)	OS (months)	Galunisertib is

[73]		galunisertib plus lomustine (79), galunisertib (39), lomustine (40)		phase II, partially blinded		plus lomustine 1 CR galunisertib 2 PR lomustine none	galunisertib plus lomustine 1.8 galunisertib 1.8 lomustine 1.9	galunisertib plus lomustine 6.7 galunisertib 8.0 lomustine 7.5	inactive
Glutamate receptors									
Iwamoto et al. 2010 [95]	Talampanel	Recurrent (22)	None	Single arm phase II	PFS-6	1 PR	PFS-6 5%	OS 3 months	Talampanel is inactive in this setting
Grossmann et al. 2009 [24]	Talampanel	Newly diagnosed, TMZ/RT→TMZ plus talampanel (72)	None	Single arm phase II	OS			OS 18.3 months	Tamlampanel may be further evaluated
CD95 ligand									
Wick et al. 2014 [96]	APG-101	Recurrent, RT (26) or RT plus APG-101 (58)	None	Randomized phase II, open label	PFS-6		PFS 6 RT 4% RT plus APG-101 21%	OS 11.5 months in both arms	APG-101 warrants further study
PD-1									
Reardon et al. 2017 [75]	Nivolumab	Recurrent, nivolumab (184) or bevacizumab (185)	None	Randomized phase III, open label	OS	Nivolumab 12 responses Bevacizumab 36 responses	Median PFS (months) Nivolumab 1.5 Bevacizumab 3.5	OS (months) Nivolumab 9.8 Bevacizumab 10.0	Nivolumab may be active in patients with MGMT promoter-methylated tumors who are not on steroids

[1]inhibits VEGFR2, EGFR, RET
[2]inhibits VEGFR2, MET, AXL

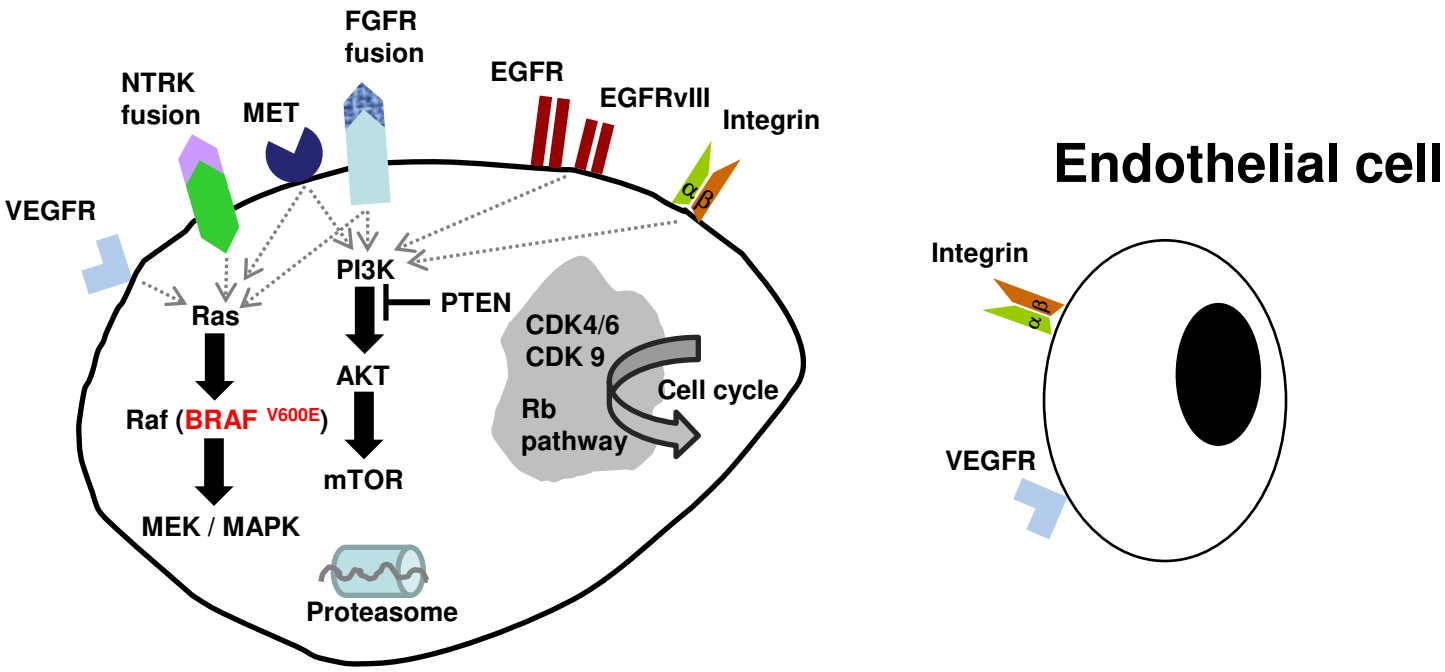
[3]inhibits VEGFR1-3, TIE2, KIT, RET, RAF1, BRAF, PDGFR, FGFR, CSF1R

Abbreviations: ND no data, OS overall survival, PCV procarbacin lomustine vincristine, PFS progression-free survival, TMZ temozolomide

Table 3. Outlook on improved drug development in glioblastoma.

Limitations and challenges	**Solutions**
Molecular testing not standardized and not reimbursed	Centralization, implementation into guidelines and proactive dialog with health care providers
Patient selection	Avoid selecting last-line patients and focus on biomarker-enriched patient populations
Insufficient drug development strategies	Early proof-of-concept (window of opportunity studies) with pharmacodynamic and biological endpoints and inclusion of glioblastoma patients in basket trials for tumors with defined molecular alterations
Administrational barriers impeding international cooperations	Parallel trials and efforts to increase public awareness in order to change legislation to facilitate international cooperation

Figure 1


Tumor cell
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NTRK fusion
MET
EGFR
EGFRvIII
Integrin
VEGFR
PI3K
PTEN
Ras
CDK4/6
CDK 9
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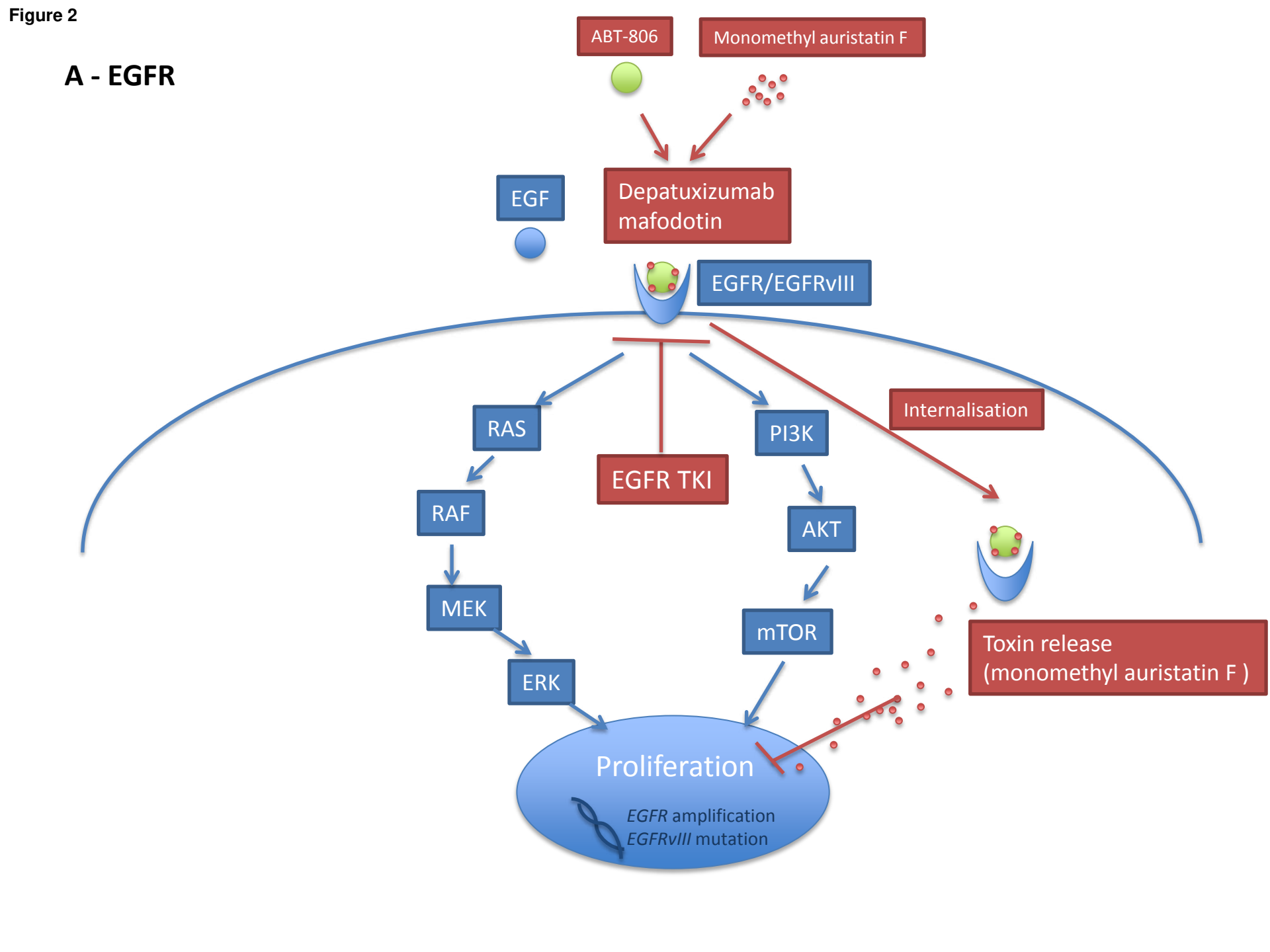
Rb pathway
AKT
Raf (BRAF V600E)
mTOR
MEK / MAPK
Proteasome
Endothelial cell
Integrin
VEGFR

Figure 2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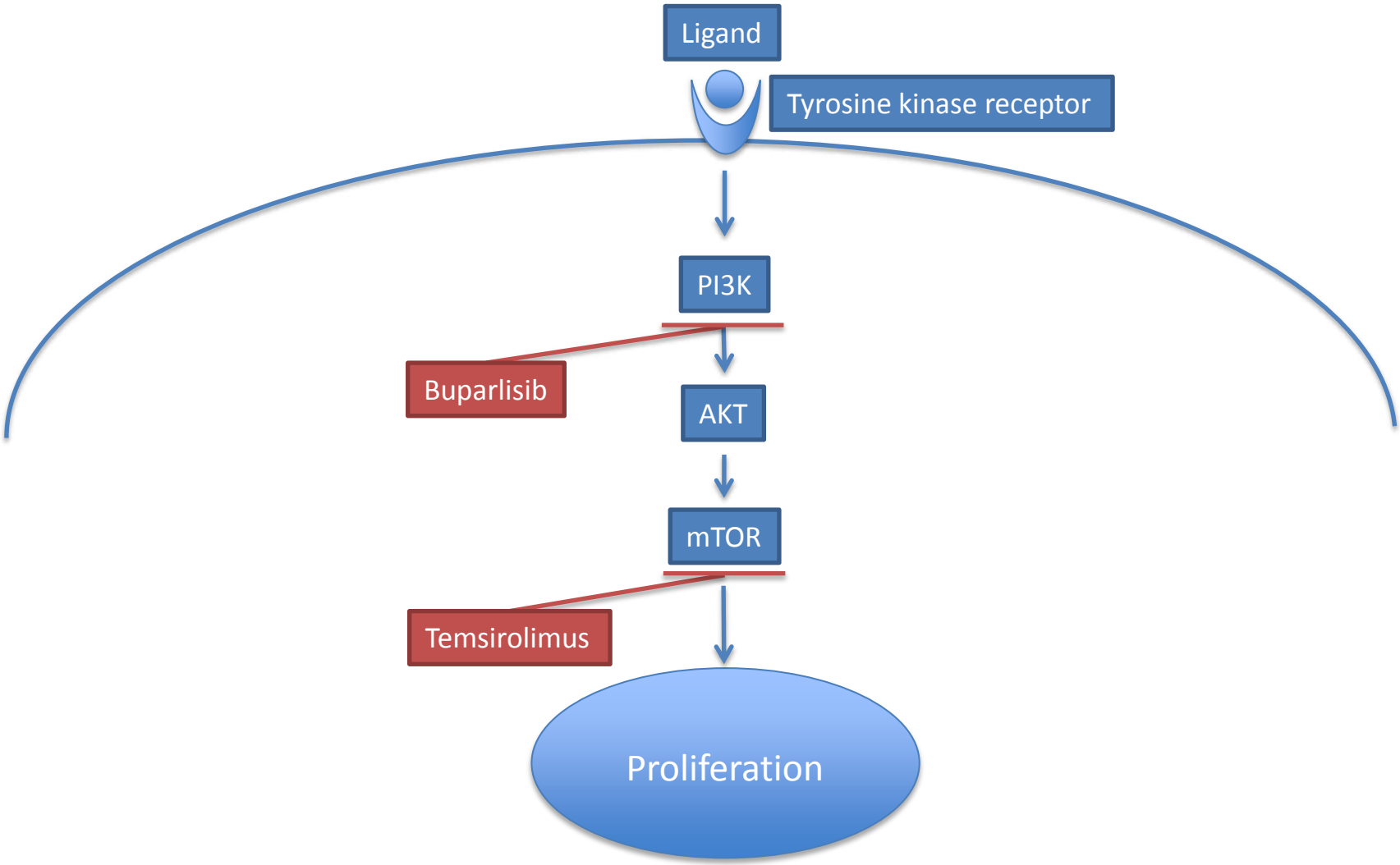
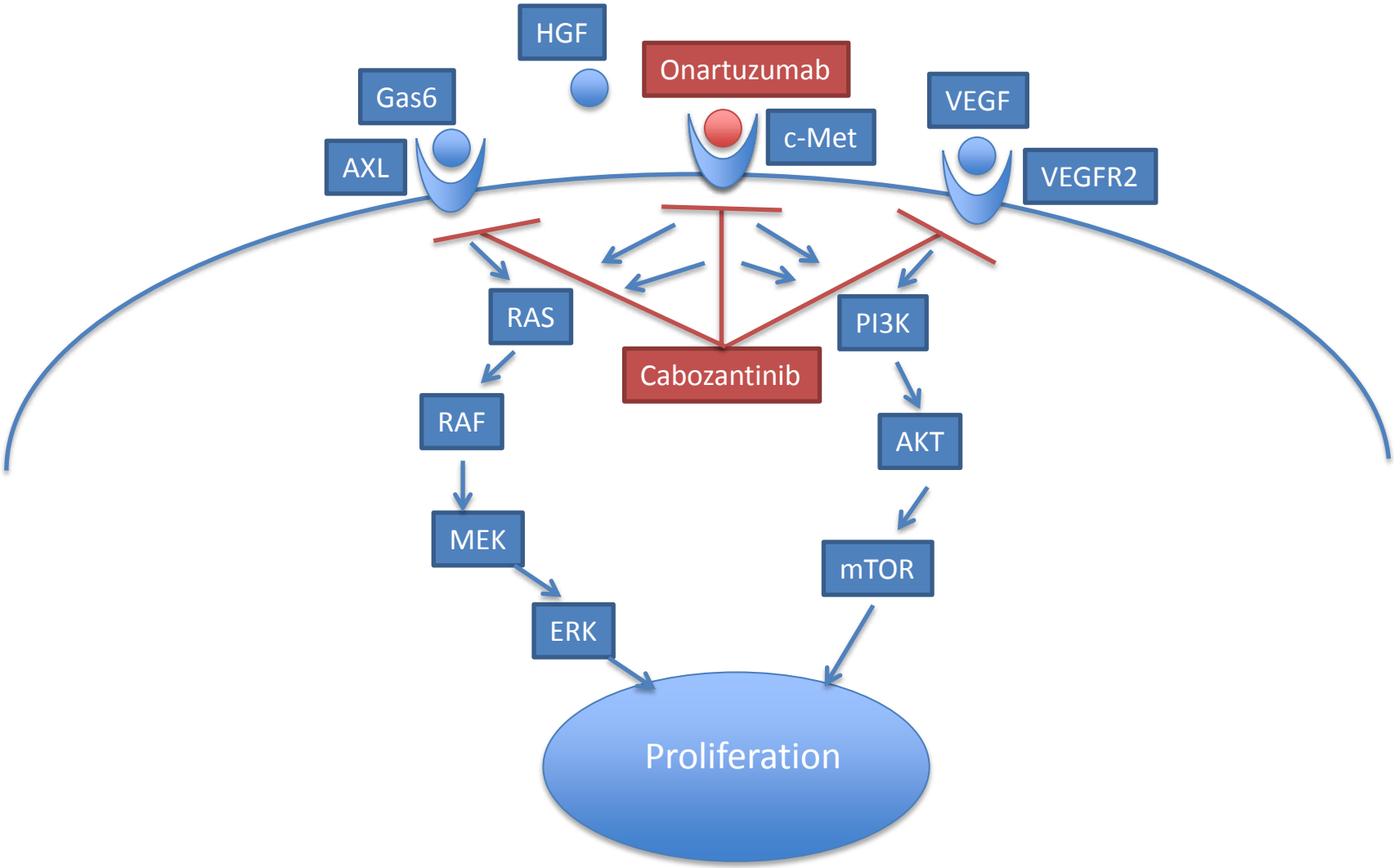
EGF
Depatuxizumab mafodotin
EGFR/EGFRvIII
RAS
PI3K
Internalisation
EGFR TKI
RAF
AKT
MEK
mTOR
Toxin release (monomethyl auristatin F)
ERK
Proliferation
EGFR amplification
EGFRvIII mutation

B - PI3K/AKT/mTor



C - MET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Gas6
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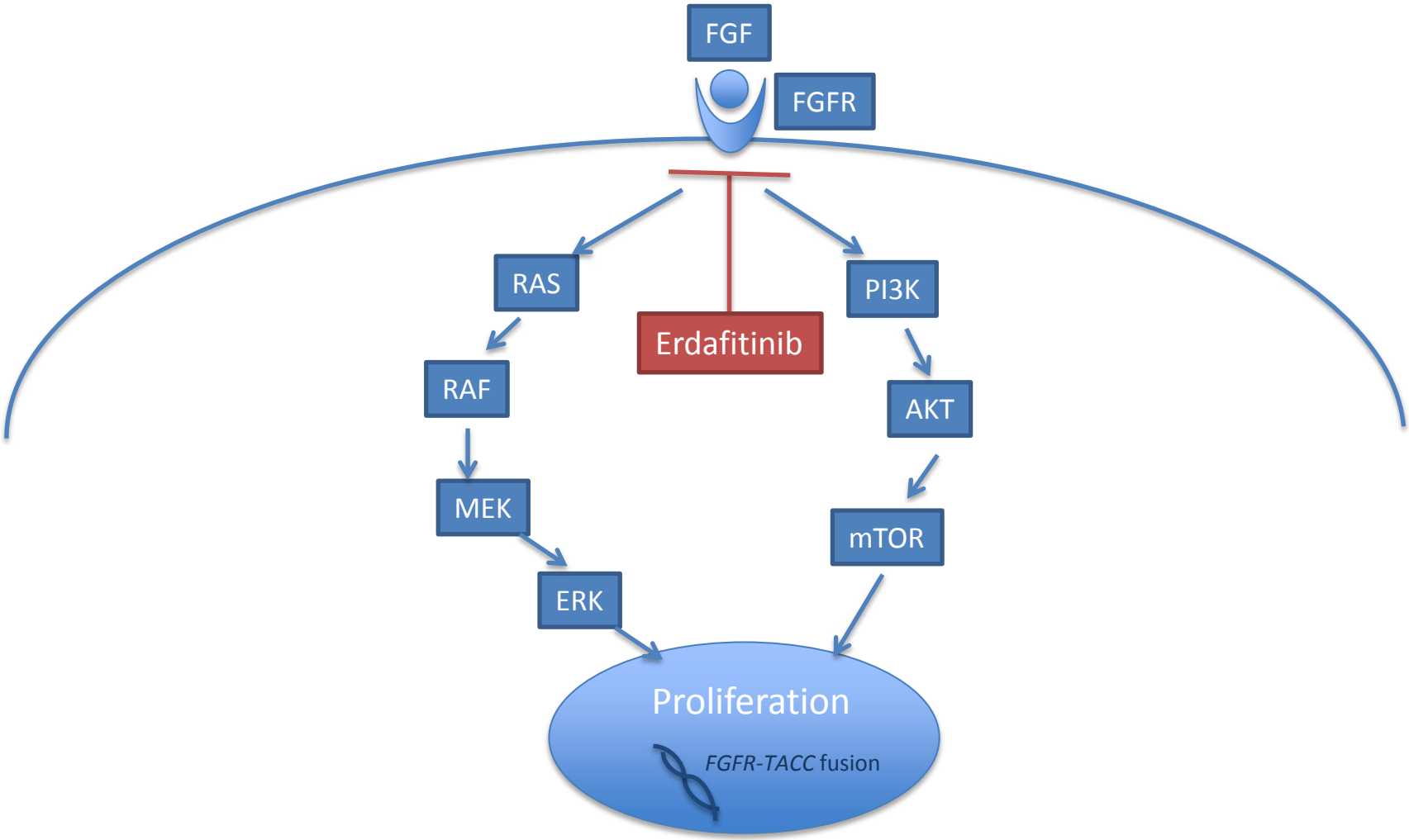
RAF
AKT
MEK
mTOR
ERK
Proliferation

D - FGFR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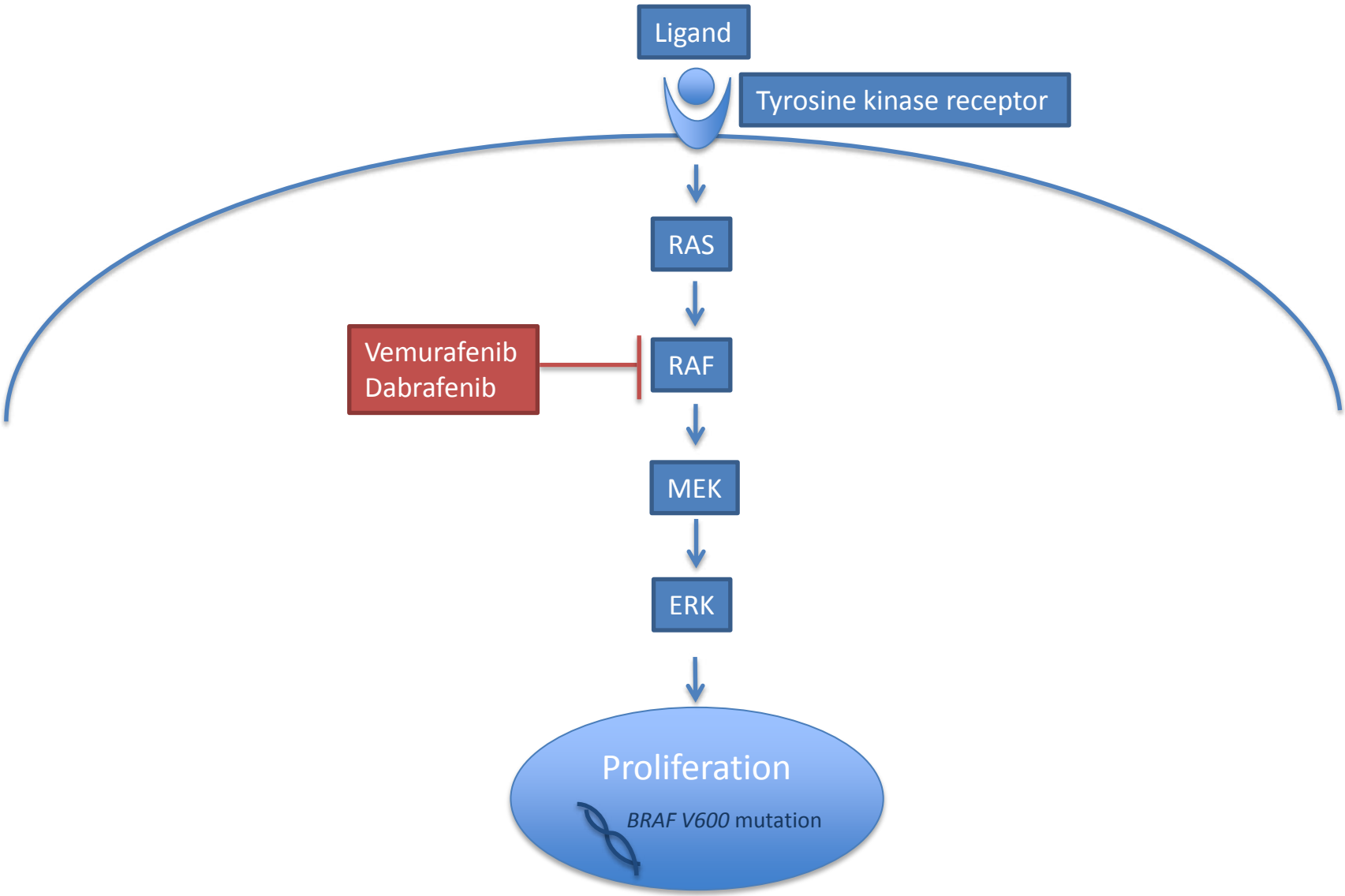
FGFR-TACC fusion

E - BRAF


Ligand
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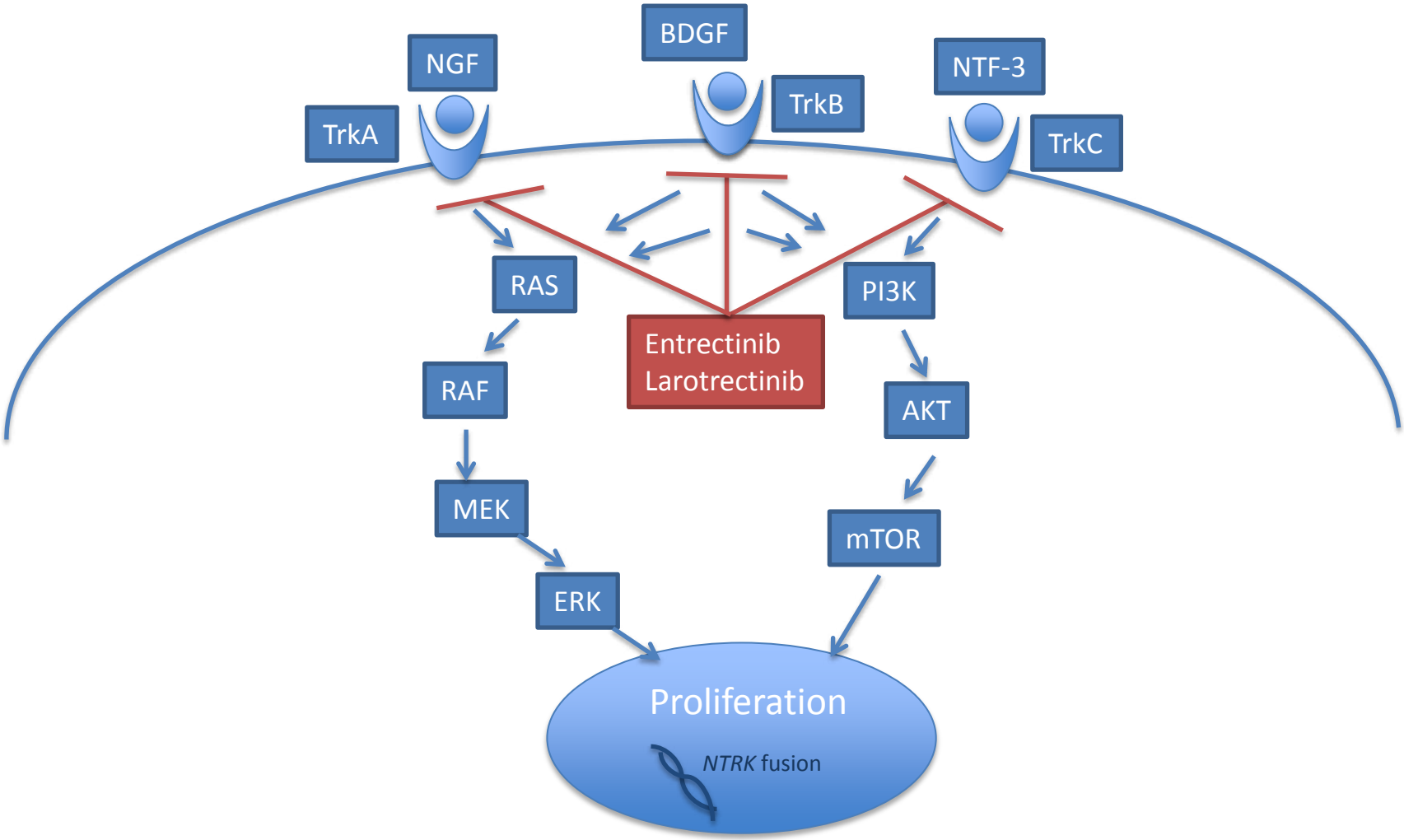
RAS
Vemurafenib
Dabrafenib
RAF
MEK
ERK
Proliferation
BRAF V600 mutation

F - NTRK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NTF-3
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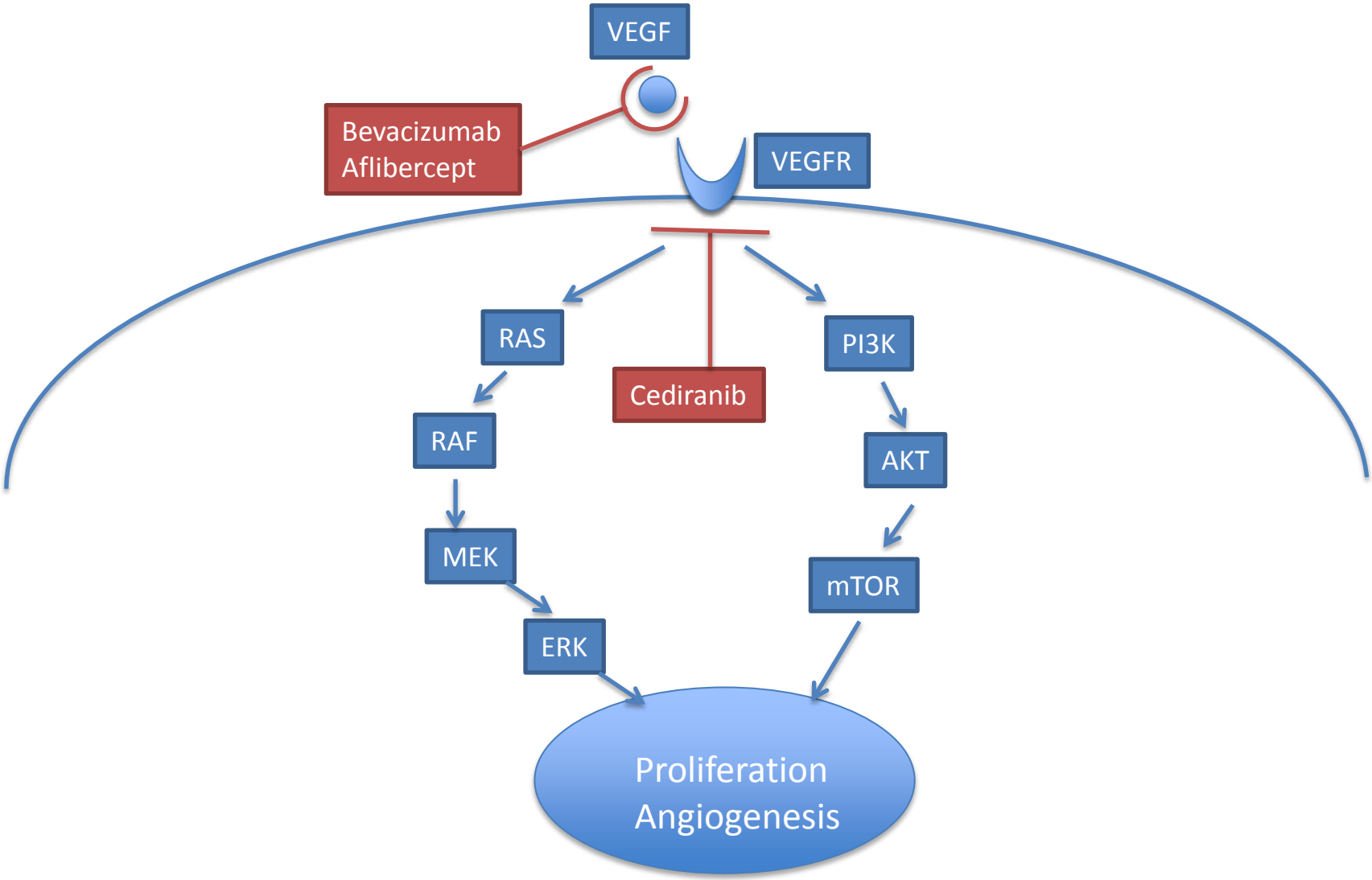
ERK
Proliferation
NTRK fusion

G - VEGF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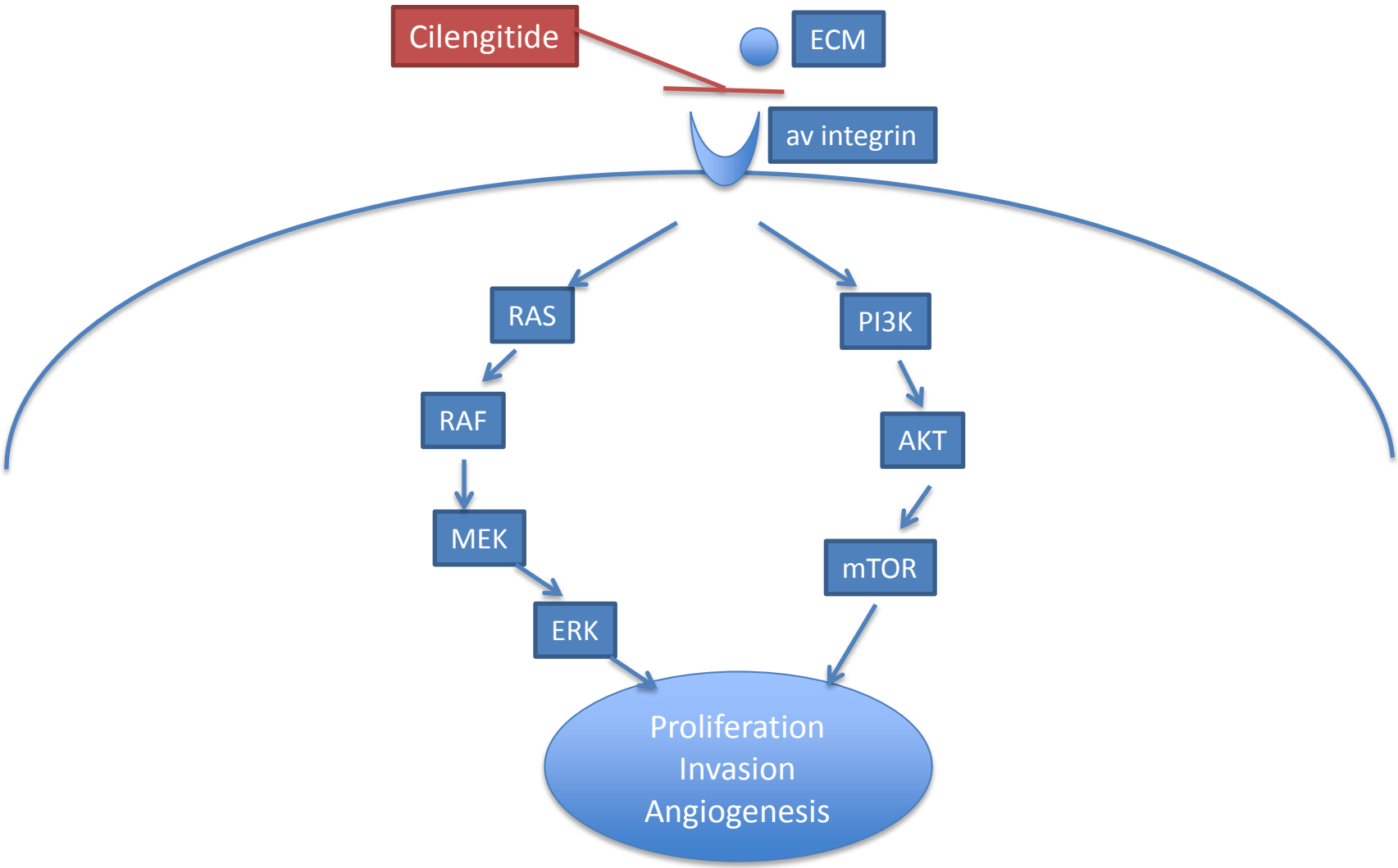
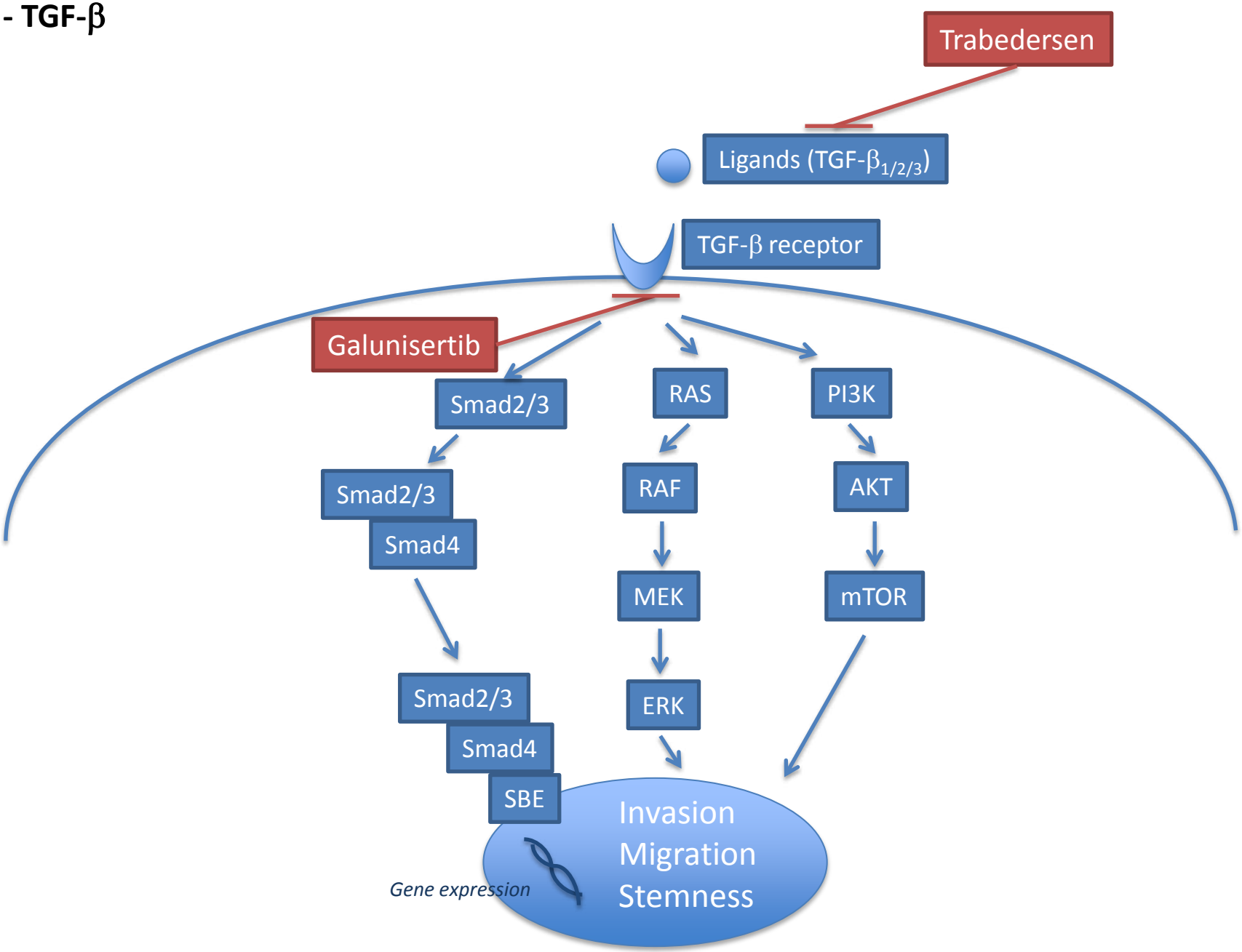
Bevacizumab
Aflibercept
VEGFR
RAS
Cediranib
PI3K
RAF
AKT
MEK
mTOR
ERK
Proliferation
Angiogenesis

H - Integrin


Cilengitide
ECM
av integrin
RAS
PI3K
RAF
AKT
MEK
mTOR
ERK
Proliferation
Invasion
Angiogenesis

I - TGF-β


Trabedersen
Ligands (TGF-β1/2/3)
TGF-β receptor
Galunisertib
Smad2/3
RAS
PI3K
Smad2/3
Smad4
RAF
AKT
MEK
mTOR
Smad2/3
Smad4
SBE
ERK
Invasion
Migration
Stemness
Gene expression